

## ABSTRACTS OF PAPERS

### Fourteenth Annual Albert L. Tester Memorial Symposium, 13-14 April 1989<sup>1</sup>

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university, and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Jonathan Roughgarden of Stanford University participated in the symposium.

### Reproductive Success of the White-rumped Shama, *Copsychus malabaricus*<sup>2</sup>

CELESTINO F. AGUON<sup>3</sup>

Reproductive success in passerine birds may be increased in a number of ways. In the White-rumped Shama (an introduced bird in Hawaii) some pairs have larger clutch sizes,

while others may raise more than one brood. Reproductive characteristics of the White-rumped Shama were studied from January 1986 to November 1987 in Makiki Valley, Oahu. Aspects of breeding biology or behavior that tend to maximize reproductive success were of particular interest. Shammas were caught in mist nets and banded with unique color and U.S. Fish and Wildlife Service bands. Nest boxes were placed in the study area where males were known or thought to be defending territories. Thirty-two nests were monitored during the study. Individual eggs

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and nestlings were uniquely marked and followed through the nesting cycle. The White-rumped Shama is a monogamous, hole-nesting species. Though only female shamas incubate and brood, both sexes feed young. Fourteen three-egg and 18 four-egg clutches were observed. The incubation period averaged 13.6 days ( $SE = 0.18$ ,  $n = 18$ ), and the nestling period averaged 12.31 days ( $SE = 0.24$ ,  $n = 13$ ). There were no significant differences ( $t$  test,  $P > 0.05$ ) between clutch sizes in incubation period ( $t_{[19]} = 1.71$ ), nestling period ( $t_{[16]} = 0.5$ ), number fledged ( $t_{[20]} = 0.48$ ), and female weight ( $t_{[16]} = 0.108$ ). Fledging success (number of eggs/number of eggs hatched) of all shama first broods was 65.7% ( $SE = 9.6$ ,  $n = 17$ ). Shammas that raised

two broods had a fledging success of 87.5% for the first brood. Total fledging success of pairs raising two broods (the mean number of young fledged from both broods divided by the mean number of young hatched) averaged 90.6% ( $SE = 0.9$ ,  $n = 4$ ), but this was not significantly different from the success rate of all first broods, possibly because sample sizes were small. Number of clutches rather than clutch size per se seems to be of primary importance in White-rumped Shama reproductive success. Results of this study suggest that individuals with two broods realize greater reproductive success than those with one and thus have greater fitness during that breeding season.

## Construction of a Genomic Library and Cloning of a Gene Encoding Phosphoenolpyruvate Carboxylase (PEPC) in Sugarcane<sup>4</sup>

HENRIK ALBERT<sup>5</sup>

PEPC catalyzes the first step of carbon fixation in C4 plants. Characterizing the structure and mode of regulation of the gene encoding this enzyme should increase our understanding of C4 photosynthesis, of particular importance in tropical agriculture. To isolate and study this gene a genomic library has been constructed for sugarcane. High molecular weight nuclear DNA was extracted from meristematic tissue of mature sugarcane (*Saccharum* hybrid variety H32-8560). Twenty kilobase fragments, generated by partial digestion with the restriction enzyme *Mbo* I and NaCl gradient fractionation, were ligated to

the *Bam* HI restriction site of the bacteriophage  $\lambda$  cloning vector EMBL4, packaged in vitro and titered on several host *Escherichia coli* strains. On the *McrB*<sup>-</sup> strain K802 the yield was  $6.4 \times 10^6$  plaque-forming units (pfu) per ml, for a total library size of  $3.2 \times 10^6$  pfu. After amplification on K802 this library was estimated to be greater than 90% recombinants. Clones ( $1 \times 10^6$ ) (approximately three genome equivalents) were screened with a radioactive probe made from a partial cDNA clone for PEPC from *Flavaria trinervia*, and 18 candidate clones were selected. Further characterization of these clones revealed that five independent clones, containing inserts ranging from ca. 12 to 18 kb, were positive for PEPC. Southern blot analysis shows that at least one restriction fragment from each clone hybridizes to the PEPC probe.

<sup>4</sup> This work was supported in part by a fellowship from Hawaii Sugar Planters' Association.

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## Sodium Channel Activation in Crayfish Axons: Effects of Deuterium Oxide<sup>6</sup>

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Schauf and Bullock (1979, *Biophys. J.* 27: 193) have reported for *Myxicola* giant axons that substitution of heavy water ( $D_2O$ ) externally and internally slows the kinetics of sodium channel ionic current ( $I_{Na}$ ) without significant change in gating current ( $I_g$ ) kinetics. In crayfish axons, I confirm Schauf and Bullock's findings: (1)  $I_{Na}$  is delayed without change in gating current kinetics or total charge movement; (2)  $D_2O$  has no apparent effect on the "Cole-Moore-type" shifts of  $I_{Na}$  rising phase; (3) sodium channel deactivation (monitored by ionic tail currents) seems to be insensitive to  $D_2O$ ; (4) in the presence of  $D_2O$ , activation of  $I_{Na}$  is delayed. However, the change in time to peak remains a constant fraction of control time to peak. I have further observed that  $D_2O$  both prolongs the "initial

delay" before the start of the  $I_{Na}$  rising phase and slows the subsequent rate of sodium channel activation. Thus the shift in peak  $I_{Na}$  is greater than the prolongation of the "initial delay." I conclude that voltage-sensitive "gating" charge movement is generated in non-conducting transitions that precede transition to the "open" or conducting state. My confirmation in crayfish axons of Schauf and Bullock's data strengthens their argument that no significant component of  $I_g$  arises in the final opening step and supports their suggestion that  $I_g$  is generated within a hydrophobic rather than a hydrophilic environment. I interpret these findings as indicating a physical and functional separation between the voltage-sensitive gating particle and the activation gate.

## Nutrient Reabsorption in the Kidney of the Crustacean *Homarus americanus*<sup>8</sup>

RACHEL D. BEHNKE<sup>9</sup>

The cell membrane physiology of L-proline reabsorption was investigated in a decapod crustacean antennal gland. Purified membrane vesicles were prepared from the apical brush border of Atlantic lobster (*Homarus americanus*) antennal gland labyrinth and bladder epithelia. Transport of proline was monitored by fluxes of radiolabeled substrate into the vesicles. The antennal gland labyrinth epithelium showed a greater capacity for  $^3H$ -proline uptake than the bladder epithelium. In detailed studies of the labyrinth epithelium,

$^3H$ -L-proline transport was found to be  $NaCl$ -dependent, with an inwardly directed  $Na^+$  gradient driving concentrative uptake of  $^3H$ -L-proline in the presence of equilibrated  $Cl^-$ , while a  $Cl^-$  gradient had no stimulatory effect in the presence of equilibrated  $Na^+$ . This suggests that  $^3H$ -L-proline transport in these cells is energetically coupled to a transmembrane  $Na^+$  gradient and perhaps catalytically stimulated by  $Cl^-$ . An inside-negative transmembrane potential difference stimulated concentrative accumulation of  $^3H$ -L-proline, strongly suggesting electrogenic transport. Kinetic analyses showed proline transport to occur through a combination of at least one carrier-dependent process ( $K_t = 0.37$  mM;  $J_m = 1.19$  nmol/mg protein/10 sec) and apparent diffusion ( $P = 0.33$  nmole/mg protein/10 sec/mM). Uptake of proline was a sigmoidal function of  $[Na^+]$  and a hyperbolic function of  $[Cl^-]$ . Hill analyses of kinetic data suggest a substrate binding stoichiometry of  $2 Na^+ : 1 Cl^- : 1$  proline.

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<sup>7</sup>Bekesy Laboratory of Neurobiology, Pacific Biomedical Research Center, and the Department of Physiology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii 96822.

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## Transport Characteristics of Crustacean Hepatopancreatic and Antennal Gland Epithelia in Primary Cell Culture<sup>10</sup>

MARK A. CATTEY<sup>11</sup>

Transport characteristics of hepatopancreatic and antennal gland epithelial cells in the lobster (*Homarus americanus*), the freshwater prawn (*Macrobrachium rosenbergii*), and the crayfish (*Procambrus clarkii*) were observed in vitro with cells maintained on a permeable filter support. Cells were removed from the respective organs by mechanical disruption or enzymatic digestion in a physiological saline solution lacking  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  ions. Subsequently the cells were suspended in a commercial growth medium and supplemented with an appropriate salt solution for the marine or freshwater environment. This study investigated the electrical properties of the tissues by measuring the electrical resistance and potential difference (PD) developed across the cell layer over time. Preliminary data from *Macrobrachium* hepatopancreatic

cultures indicate a resistance of ca. 15 ohm/cm<sup>2</sup> and a PD of 0.7 mV. Monolayers attached to the filter supports were placed within an Ussing chamber containing saline and the net flux of radiolabeled D-glucose was measured to examine the absorptive characteristics of the different tissues. *Homarus* hepatopancreatic cultures showed net absorption of 1.0 mM <sup>3</sup>H-glucose of 1.3 nmol/cm<sup>2</sup>/min with a decrease to 0.3 nmol/cm<sup>2</sup>/min after the addition of 10 mM unlabeled glucose to the mucosal surface. Phloridzin (0.5 mM), a known inhibitor of glucose transport, also blocked 1.0 mM glucose flux (2.3 nmol/cm<sup>2</sup>/min before and 0.6 nmol/cm<sup>2</sup>/min after addition of the inhibitor). Preliminary data indicate that crustacean epithelial monolayers may be utilized to study transcellular nutrient transport.

## Identification, Characterization, and Phosphorylation of Gap Junction-Associated Proteins in Uninfected and Rous Sarcoma Virus-Infected Fibroblasts<sup>12</sup>

DAVID S. CROW<sup>13</sup>

Microinjection studies show that when the src oncogene product (pp60<sup>v-src</sup>) is active, fibroblast gap-junctional communication ceases to exist. Loss of communication may be due to phosphorylation of gap junction pro-

teins (connexins) by the tyrosine kinase activity of pp60<sup>v-src</sup>. To examine this, the nature of the connexins in fibroblasts was determined. Both uninfected and Rous sarcoma virus (RSV)-transformed fibroblasts contained mRNA identical in size to that in heart (3.0 Kb). Uninfected and RSV-transformed fibroblast connexins were detected with heart connexin43 antibody by western blotting and immunoprecipitation. Uninfected fibroblast connexins had molecular weights of 43, 45, and 47 Kd, like the 45- and 47-Kd connexins from heart tissue. Connexin from RSV-transformed fibroblasts had a molecular weight of 43 Kd. Peptide maps of the 43-Kd connexin from both the uninfected and RSV-

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transformed fibroblasts were identical. Phosphorylation studies showed that the 43-, 45-, and 47-Kd proteins from uninfected fibroblasts are phosphorylated on serine, while the 43-Kd protein from RSV-transformed fibroblasts was phosphorylated on both serine and tyrosine. Treatment of immunoprecipitated connexins from the uninfected fibroblasts with serine-specific phosphatase resulted in a

loss of the 47- and 45-Kd species and an increase in the 43-Kd protein. Thus, loss of communication seen in RSV-transformed fibroblasts is not due to a lack of gap junction protein; instead, it may be related to a decrease in the serine phosphorylated 45- and 47-Kd species caused by tyrosine phosphorylation of the 43-Kd protein.

## Observations on the Social Behavior of a Small Population of the Butterflyfish *Chaetodon ornatissimus* in Kaneohe Bay, Hawaii

G. CURT FIEDLER<sup>14</sup>

Since May 1988, the population of *Chaetodon ornatissimus* on a patch reef (reef no. 41) in Kaneohe Bay has been monitored. These fish are territorial corallivores and live in heterosexual pairs. Monogamy in fish is rare, but among chaetodontids, this seems to be the rule. Observations of *C. ornatissimus* in groups larger than two provides an interesting comparison. The work presented here is a portion of an ongoing project to study this butterflyfish. Assessment of the population was easy, as they are relatively conspicuous, few in numbers, and exhibit large home ranges. Home ranges were arranged parallel to the circumference of the reef. Identification of individual fish was possible because of the slight variation in tail and body striping that retains its character over months. Beginning in August, measurement of adjacent territories was attempted. Counts of social groupings, chases, and behavior during feeding were recorded as well. Descriptions of modal action patterns were based on underwater video. Underwater photography of individuals was employed to obtain a record of individual markings. Adjacent reefs were also surveyed for *C. ornatissimus*. A group size of two indi-

viduals was the most common, with six being the maximum. In observations of two fish, pairmates occurred together more often. Within one particular unstable trio, one individual was seen associated with two others somewhat equally. Movement data indicate that feeding pairmates oriented movement mostly toward each other, with one individual taking the lead more often. Intrapair chases were one-sided, indicating that one individual was almost always the aggressor and foraging leader. An index of aggression is calculated from chase data for each individual. The order of individual aggression indices closely followed the order of sizes. Differences in agonism did not occur between morning and afternoon observations. Over the summer and fall of 1988, it was observed that *C. ornatissimus* recruited to reef no. 41 as adults and juveniles. An abrupt disappearance of large adults was noted in February. Data suggest that within *C. ornatissimus* pairs there is intrapair dominance, as well as a dominance hierarchy among all individuals on the reef, based on sex and size. Sharing of pair-mates in this species seems to be possible in this habitat. Counts of *C. ornatissimus* on patch reefs indicate a previously unsuspected widespread distribution within northern Kaneohe Bay. Stability of an assemblage of *C. ornatissimus* on these patch reefs over months is suspect.

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## Calcium Current and Veiling Neuronal Outgrowth: A Relationship?<sup>15</sup>

ROBERT GRAF<sup>16</sup>

A unique feature of crustacean peptidergic neuron outgrowth in culture is that it occurs quickly in defined medium. It has been postulated that this immediate outgrowth may utilize the exocytotic mechanism underlying neurosecretion (Cooke, Graf, Grau, Haylett, Meyers, and Ruben, Proc. Natl. Acad. Sci. U.S.A. 86:402-407). I have been accumulating data that bear on this hypothesis using neurosecretory cells from the X-organ-sinus gland system of the land crab, *Cardisoma carnifex*. The neurons studied (soma diameter 30-50  $\mu\text{m}$ ) exhibited broad lamellopodial (veiling) outgrowth from the distal axonal process. Veiling outgrowth was found to be severely inhibited when cultures were grown in medium containing  $10^{-4}$  M  $\text{CdCl}_2$ . The whole-cell patch configuration was used for voltage clamping large veiling neurons. These show prominent inward current from record-

ings made at the soma. Inward currents include a rapidly activating and inactivating current blocked by TTX ( $10^{-7}$  M), thereby identified as a Na current, and a current blocked by  $\text{CdCl}_2$  ( $10^{-4}$  M), attributed to Ca. Ca current remained unchanged at holding potentials between -40 and -60 mV, was observable for depolarizing steps greater than -30 mV, and was maximal at +10 mV. Its magnitude was up to 1 nA, and minimum time to peak was ca. 5 msec. Decay during steps to +10 mV showed an initial rapid phase (<10 msec) and a much slower one (>50 msec); at 160 msec, more than 50% of peak current remained. My observation that inhibition of Ca current blocks outgrowth supports other work in this area and joins it with previous research linking Ca current to exocytotic secretory mechanisms.

## Trophic Relationships of Deepwater Snappers (Lutjanidae) from Penguin Bank, Hawaii<sup>17</sup>

WAYNE R. HAIGHT<sup>18</sup>

The combined area of the major Hawaiian deepwater banks is over five times that of all shallow-water reef systems in the archipelago. Nearshore reef fish trophics have been extensively studied, but there has been no focused study of the deepwater banks that would permit understanding the ecology of commercially important fishes. The objective of this study

was to identify and characterize relationships in prey utilization, depth distribution, and feeding interval between commercially important deepwater snappers on Penguin Bank. Two hundred eighteen gastrointestinal samples representing six deepwater snapper species were examined. Analysis of prey use indicates that these species are separated by diet into two feeding guilds: (1) species feeding primarily on zooplankton (salps and pteropods), and (2) species feeding primarily on larger prey (fish). Zooplankton composed 88.8%, 97.2%, and 53.0% of the total index of relative importance (IRI) in the diets of the *Pristipomoides* species, *P. filamentosus*, *P. sieboldii*, and *P. zonatus*, respectively. The *Etelis* species, *E. carbunculus* and *E. coruscans*, along with *Aprion virescens* were

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primarily piscivorous, with fish making up 96.5%, 78.3%, and 87.3% of their respective total IRI. Diets of all species within each of these guilds overlap, but very little dietary overlap exists between species in different feeding guilds. Feeding time of day and depth were significantly different for all species within the piscivorous guild. Feeding time did

not differ significantly between species within the zooplanktivorous guild; however, *P. filamentosus* fed at a significantly shallower depth than the other two *Pristipomoides* species. These results suggest that trophic competition is unlikely between lutjanid snapper feeding guilds on Penguin Bank.

## Biological Effects of Ultraviolet Radiation

TINA HAZZARD<sup>19</sup>

Increased depletion of the protective ozone layer through the release of chlorofluorocarbon gases has created an urgent need to understand the biological effects of ultraviolet (UV) radiation. Although some animal tissues would be damaged by an increase in UV radiation, the greatest global concern should be the potential inhibition of the ability to trap light and fix atmospheric carbon by primary producers in an UV-enriched environment. In situ responses to ambient levels of UV and the attenuation of UV radiation at various depths were examined in the summer and fall of 1988 in Kaneohe Bay through incubations of naturally occurring phytoplankton and the utilization of a chemical actinometer. The attenuation of UV radiation into the water of

Kaneohe Bay fits a log linear relationship where 50% of the UV is absorbed within the first 2.8 m. Net primary productivity of UV-exposed algae was found to be significantly less than that of the control group when incubated at 0, 1-, and 2- m depths, but no significant difference was found directly at a depth of 4 m. Incorporation of radioactive carbon into proteins, lipids, intermediate weight compounds, and polysaccharides was found to be inhibited in approximately equal proportions at each depth, which suggests that the action of UV inhibition is in the light-trapping steps of photosynthesis and not in the pathways of carbon assimilation.

## Mitochondrial DNA Used to Track the Dispersal of a Preferred Menu Item in Waikiki Restaurants: Mahimahi as an Example of Genetic Tagging with mtDNA

CHRISTOPHER E. HERZIG<sup>20</sup>

International predation pressure on pelagic marine species in Hawaii has created concern about the possibility of overfishing in a variety

of large-bodied and long-lived fish. Mahimahi (*Coryphaena hippurus*) has a world-wide distribution in tropical and subtropical waters, but precise identification of separate stocks has proven problematic using conventional starch gel electrophoretic methods. As an attempt to develop genetic methods for tagging these animals, mtDNA sequences were amplified via the Polymerase Chain Reaction

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(PCR) from total genomic DNAs prepared from larvae, fingerlings, and fin-clip samples of a tank-reared population used for aquaculture development. In a comparison of about 330 bp of 12S rRNA sequence, all inbred fish examined ( $n = 3$ ) were found to be identical. Comparison of 201 bp from the cytochrome b gene reveals sequence differences between mahimahi caught in Hawaii versus those from Taiwan. Presently we are sequencing samples of wild-caught mahimahi from different geo-

graphic areas of the Pacific. In an attempt to find more intraspecific polymorphism, we are also working on amplifying via PCR the D-loop region of the mtDNA. If sequence differences can be found, then specific probes could be designed to identify different stocks. The ability to screen large samples of wild-caught fish with stock-specific probes will greatly improve the understanding of population structures.

## Why Do Tunicate Larvae Settle Only during the Daytime?<sup>21</sup>

CATE HURLBUT<sup>22</sup>

For sessile marine invertebrates with short-lived larvae, the daily time of larval release into the plankton can determine environmental cues available to larvae, which can affect settlement location and hence juvenile survival. I examined the diel pattern of larval release of the colonial tunicate *Didemnum candidum* by counting larvae released hourly from adults placed in traps, sampling larvae in the plankton every 2 hr using a pump, and determining settling times on plates immersed for 2-hr periods. All three methods yielded a similar diel pattern of larval release and settlement. Larvae and settlers were only present during the daytime, with strong early morning and

weaker afternoon peaks in abundance. Because release only occurred during the daytime, larvae were settling when light was available as a cue. To examine the effects of light level on settlement, I documented larval phototaxis in the laboratory and compared both settlement and survival in the field on light and dark surfaces. Larvae settled photonegatively in both the laboratory and the field. Settlement and survival were highest on the undersides of opaque plates where settlers and juveniles were protected from direct light. Light is apparently important to larvae in locating settlement sites where juvenile survival is maximized.

## Effects of Photoperiod and Temperature on Ovarian Maturation in the Striped Mullet, *Mugil cephalus*<sup>23</sup>

CHRISTOPHER D. KELLEY<sup>24</sup>

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In Hawaii, the annual reproductive season of the striped mullet, *Mugil cephalus*, extends between September and March. Correlation of the onset of oocyte maturation stages with photoperiod and temperature changes during this period suggest that the cortical vesicle stage and the vitellogenic stage may be regulated independently. This study was carried out to test that hypothesis, which has not been addressed in previous studies on the environmental control of fish reproduction. A

total of 132 female mullet was used in three 8-week trials during 1988. A similar protocol was used for each trial. Between 36 and 48 fish were maintained under four combinations of photoperiod and temperature (8 hr light at 21°C, 8 hr light at 30°C, 16 hr light at 21°C, and 16 hr light at 30°C). Oocyte samples were obtained from each fish on 2- to 4-week intervals and examined under a microscope to determine the stage of maturation. Trial 1 was started in April when half of the females had primary growth stage oocytes while the other half had cortical vesicle stage oocytes. Trial 2 was started in July when all females had only primary growth stage oocytes, and trial 3 was started in September when all of the females had cortical vesicle stage oocytes. Results from trial 1 indicated that mullet have a postseason refractory period. Vitellogenesis was not initiated in 8 weeks under any environmental

conditions. Results from the other two trials supported the hypothesis. In trial 2, both stages of maturation were initiated under a combination of short photoperiod and low temperature. Both stages were inhibited under a combination of long photoperiod and high temperature. The cortical vesicle stage was initiated while the vitellogenic stage was inhibited under a combination of short photoperiod and high temperature. In trial 3, vitellogenesis was inhibited in over 50% of the females under short photoperiod and high temperature, but was not inhibited under a combination of long photoperiod and low temperature. These data suggest that photoperiod is the primary factor determining onset of the cortical vesicle stage and water temperature is the primary factor determining onset of the vitellogenic stage in this species.

## Concurrent Speciation in Temperate Sea Urchin Lineages<sup>25</sup>

B. D. KESSING<sup>26</sup>

Members of the temperate subfamily Strongylocentrotinae, the most widely studied group of sea urchins, have broad distributions and extensive larval dispersal. They are, therefore, expected to speciate infrequently. Unfortunately, the pattern of species formation in this group is, as with many marine invertebrates, poorly known because of their inadequate fossil record. It is possible, however, to examine the evolutionary history of species poorly represented in the fossil record by using molecular techniques. Phylogenetic relationships and patterns of speciation for seven species of sea urchins in the subfamily Strongylocentrotinae were explored by comparing mitochondrial DNA (mtDNA) sequence data. The Polymerase Chain Reaction was used to amplify three protein-coding

regions from the mtDNA of seven species of strongylocentrotids (*Strongylocentrotus pallidus*, *S. purpuratus*, *S. droebachiensis*, *S. nudus*, *S. franciscanus*, *S. intermedius*, and *Hemicentrotus pulcherrimus*). For all species, 800 nucleotides were sequenced using the Sanger dideoxynucleotide method. Silent substitutions at third codon positions were compared among species to determine phylogenetic relationships using both parsimony and genetic distance methods. Two ancestral lineages diverged early in the evolution of the subfamily Strongylocentrotinae. Assuming similar rates of nucleotide substitutions, all seven species diverged during a speciation window representing less than 15% of the time since the ancestral lineages formed. Speciation did not occur in the period represented by the first 45% or the last 40% of time since the divergence of the ancestral lineages. These results suggest that these species went through almost concurrent speciation midway through their evolutionary history.

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## Sexual Variation in Dry Weight, Carbon and Nitrogen Content, and Fecal Pellet Production of the Copepod *Scottocalanus securifrons*<sup>27</sup>

ANN E. HILLMANN-KITALONG<sup>28</sup>

The potential nutrient input by vertically migrating copepods to deeper waters is important in understanding the trophic dynamics of the pelagic community. The calanoid copepod *Scottocalanus securifrons* was collected from 586 m at Keahole Point, Hawaii, during March and July 1988. Copepods were either immediately frozen for laboratory analysis of dry weight and carbon and nitrogen content, or used in fecal pellet production experiments. In the fecal pellet production experiments, copepods were placed inside a covered cardboard box with a 6 by 6 array of 20-ml shell vials and then placed in a refrigerator at their ambient temperature (6–7°C). Fecal pellets were counted at variable time intervals after each collection. *Scottocalanus securifrons* had a mean dry weight of  $1.00 \pm 0.20$  mg (mean  $\pm$  SD) that tended to be higher for females than males during March. The carbon to nitrogen ratios were ca. 4:1, which

is similar to those of other copepod species at this depth and latitude. Carbon and nitrogen contents were similar for males and females, regardless of the month collected. Fecal pellet production rates tended to be higher for females (1.5 fecal pellets/h) than for males (0.89 fecal pellets/h), regardless of month sampled. *Scottocalanus securifrons* fecal pellets were larger than pellets of other copepod species. The pellet dry weight was  $2.82 \pm 0.40$   $\mu$ g and contained  $0.45 \pm 0.09$   $\mu$ g carbon (17% of the dry weight of the pellet and 0.05% of the dry weight of a copepod). Fecal pellets produced about  $1.8 \times 10^3$  bacteria colonies, containing white (60%), yellow (10%), and red (30%) colonies. The sinking rate of several pellets averaged  $164 \pm 60$  m/day. These values suggest that the *Scottocalanus* population at 586 m may be an important contributor of carbon, nitrogen, and bacteria at that depth.

## Population Dynamics of Insectivorous and Nectarivorous Hawaiian Honeycreepers (Fringillidae, Drepanidinae)<sup>29</sup>

JAAN K. LEPSON<sup>30</sup>

Population dynamics of forest birds were studied at Hakalau Forest National Wildlife Refuge on the island of Hawaii. Over 1000 individuals of eight native species were cap-

tured and banded during 1987 and 1988 within a system of 10 mist nets in an 8-ha study site. Many individuals were recaptured numerous times, and the capture process does not seem to cause mortality or site abandonment by the birds. The proportion of adult birds captured in 1987 that were recaptured in 1988 was fairly even for all but the rarest species. This, along with the lack of correlation between adult recapture rate and overall abundance, indicates that breeding success, measured as proportion of hatch-year birds, could be directly compared among species varying in diet and abundance. This study compared five species of Hawaiian honey-

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creepers, including two nectarivorous and three insectivorous species. Data from June and July of both years were used because of equal sampling effort during these months, which immediately follow the breeding season. Categorical modeling procedures revealed that the proportion of hatch-year birds did not vary significantly between years except for

insectivorous honeycreepers. Adult capture frequencies were stable, indicating that the greatly reduced breeding success was not associated with lower adult population size. Decreased breeding success of insectivorous honeycreepers occurred in 1988, a year associated with drier weather, potential insect competitors, and disease.

## Community Analysis of Benthic Marine Algae in Southern China<sup>31</sup>

JANE E. LEWIS<sup>32</sup>

A field study was conducted in southern China from autumn 1986 to summer 1987 on distributions of benthic marine algae. With the assistance of a team of taxonomists specializing in various of the groups collected, preliminary identifications were assigned to

all specimens. Using this information, community analyses were conducted utilizing the COENOS and Cornell Package software programs. Results of community analyses are discussed in relation to oceanographic features of the area and seasonal changes in the flora.

## Density Alters the Form of Intraspecific Encounters in *Penaeus vannamei*

JEFFREY L. MAHON<sup>33</sup>

Behavioral response to stocking density by a species being raised for aquaculture may adversely affect the yield. *Penaeus vannamei* is often raised at very high stocking densities, but the effects on behavior have not been investigated. *P. vannamei* weighing between 17 and 20 g were observed in a simulated raceway at densities of 50, 100, and 150 individuals/m<sup>2</sup>. These densities range from higher extensive cultures to lower intensive cultures. The number of front-to-front encounters be-

tween shrimp stayed constant at all three densities. However, the front-to-side and front-to-rear encounters increased at a rate greater than predicted by density from 50 to 100 individuals/m<sup>2</sup> and increased at the rate predicted by density from 100 to 150 individuals/m<sup>2</sup>. Any random-encounter model predicts an increase in the number of encounters as density increases. It appears that the shrimp avoided an increase in front-to-front encounters much like some highly social vertebrate species. This could be due to the higher danger involved in front-to-front encounters where the opposing shrimp's chelipeds are met. Such socially adaptive behavior could account for the relatively high yield of this species at very high densities. When selecting a species for high-density aquaculture, this characteristic could facilitate attainment of high yields.

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## Pesticidal Activity of Extracts of the Endemic Hawaiian *Zanthoxylum* L. (Rutaceae)<sup>34</sup>

KEN MARR<sup>35</sup>

Many species of the genus *Zanthoxylum* contain insecticidal compounds. These include alkaloids, amides, and coumarins. Worldwide there are ca. 270 species; most are tropical. In Hawaii, four species are currently recognized. Hawaiian species have not been evaluated for pesticidal activity. Leaves, and if available, fruit, were collected from 99 trees of three species from 22 locations on Kauai, Oahu, and Hawaii. A fruit fly (*Dacus dorsalis*) egg bioassay was utilized to screen the hexane extract of leaves and pericarp for insecticidal activity. Gas chromatography (GC) and GC-mass spectroscopy were used to identify several compounds. A wide range in toxicity was found at all levels of comparison, between species, between and within populations, and between tissues. Toxic extracts of *Zanthoxylum kauaense* and *Z. hawaiiense* contained relatively high concentrations of 2-

undecanone and 2-tridecanone. Toxic extracts of *Z. dipetalum* contained high concentrations of anethole. Pure samples of these compounds were also toxic. Although these compounds are known to be insecticidal, they have not been implicated in the insecticidal activity of *Zanthoxylum* species. Pericarp extracts from five individuals of one population showed a high degree of variation in the concentration of 2-undecanone and 2-tridecanone, as well as other major constituents. Variation in quantity and quality of insecticidal compounds between trees (of one species) of a single population may have an adaptive advantage for insect resistance. Intraspecific chemical variation should also be an important consideration in agriculture and forestry. Characterization of the chemistry of a species should be based on several collections.

## Molecular Evolution in Sharks<sup>36</sup>

ANDREW MARTIN<sup>37</sup>

The documentation of variability in the rate of molecular evolution between different taxa has profound implications bearing on our understanding of mechanisms governing change in DNA sequences. Preliminary analysis of mitochondrial DNA from 10 species of sharks with known divergence times indicates that the rate of DNA divergence is substan-

tially lower than that estimated for higher vertebrates. Endonuclease restriction site analysis reveals that there is a higher proportion of shared restriction sites between shark taxa than predicted based on similar analysis of higher vertebrates. Estimated average divergence rates for silent and replacement nucleotide positions obtained using the polymerase chain reaction and direct DNA sequencing are 1.9% and 0.04% per million years for sharks, respectively. In contrast, estimated rates for primates are 9.4% and 2.0% per million years. One reason for the low observed rate of DNA divergence in sharks may be due to their utilization of urea as an osmolyte. A consequence of this strategy is that urea concentrations are sufficiently elevated (up to 700 mM) to perturb enzyme

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structure and function. It is thought that there is strong selection on protein conformation imposed by urea; therefore, the rate and types of amino acid changes allowable may be

severely restricted in sharks relative to mammals. Experiments are currently in progress to assess whether urea may influence the rate and directionality of DNA divergence.

## Rapid Evolution of Reproductive Isolation by Gamete Incompatibility in Hawaiian Sea Urchins<sup>38</sup>

EDWARD C. METZ<sup>39</sup>

Sea urchins of the genus *Echinometra* are abundant in mixed populations of two color types in Hawaii. They are remarkably similar in morphology and ecology, and opinions on whether or not they are distinct species have differed. Cross-fertilizability of gametes between the two color types is low, suggesting that they have become reproductively isolated in spite of their similarity. Genetic relationships and gamete interactions between the Hawaiian *Echinometra* have been characterized because this system holds promise for the study of marine speciation. The relationship of *Echinometra* color types was estimated by comparisons of mitochondrial DNA (mtDNA) and single copy nuclear DNA (scnDNA). Restriction endonuclease fragment patterns of mtDNA revealed two distinct genotypes, 2.0% divergent, that correspond exactly to the two color types. To estimate the relatedness of nuclear genomes, thermal stability of reassociated scnDNA hybrids was measured.<sup>40</sup> DNA hybrids within a

color type were an average of 1.8°C more thermostable than DNA hybrids between color types. On this basis the color types possess distinguishable nuclear genomes. Measurement of cross-fertilizability showed that even when sperm concentration was raised to three times that required for 100% fertilization within a color type, only 1–2% fertilization was achieved between color types. Evidence for contemporaneous spawning and developmental compatibility of these sea urchins points to the importance of fertilization itself in maintaining isolation between them. Based on reproductive isolation and genetic distinctness, the two Hawaiian *Echinometra* color types are two separate species. Comparison with other echinoid species pairs shows that this pair is much more closely related and less cross-fertilizable. Accordingly, gamete incompatibility has arisen rapidly between the Hawaiian *Echinometra* species. This isolating mechanism may be important in speciation of high-dispersal organisms.

## Ecological Determinants of Nest Location in the Laysan Finch<sup>41</sup>

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<sup>38</sup>Supported by NSF and the RCMI program of NIH.

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The endangered Laysan Finch (*Telespiza cantans*) is a little-known member of the Hawaiian honeycreeper subfamily, which is endemic to the Hawaiian Islands. This finch occurs naturally only on Laysan Island, although an introduced population occurs on Pearl and Hermes Reef. From 1986 to 1988 I studied aspects of the finch's breeding biology in situ to better understand the ecology of this species as well as to clarify management needs for its continued long-term survival. Wooden

nest boxes were placed on Laysan Island and Pearl and Hermes Reef in an attempt to facilitate the collection of clutch data. In addition, natural finch nests were located during the breeding season; various aspects of the nest, such as plant substrate, were recorded. Vegetation surveys show that vegetation components differ among Laysan and the various islands of Pearl and Hermes Reef. Results

indicate that finch nest placement is heavily dictated by the available numbers and types of plant species and suggest that other factors such as environmental temperatures, wind, precipitation, and other bird species may also modify nest placement. Maintaining the native ecosystem on Laysan appears to be a very important management consideration for the long-term survival of the Laysan Finch.

### Possible Trophic Links in Penaeid Shrimp Aquaculture Ponds<sup>43</sup>

SHAUN M. MOSS<sup>44</sup>

Natural pond biota have been implicated as being important prey items for penaeid shrimp grown in semi-intensive and intensive aquaculture ponds. Because most prey items normally consumed by shrimp in their natural habitats become rare or absent in these systems, organisms at lower trophic levels (i.e., bacteria, algae, protozoans, and meiofauna) and microbial-detrital aggregates are assumed to contribute to shrimp growth. Their trophic interactions in aquaculture ponds and their relative importance to shrimp growth is unknown. An experiment was designed to determine the effects of selected size fractions of pond water, containing potential prey items, on shrimp growth. *Penaeus vannamei* were stocked at 40/m<sup>2</sup> in replicate microcosm tanks supplied with flow-through pond water that was size fractionated to exclude specific particulate and dissolved water-column fractions. Shrimp growth in unfiltered pond water was compared with that in 5.0- $\mu$ m-filtered

pond water, 0.5- $\mu$ m-filtered pond water, activated carbon-treated pond water, and a well water control. Biotic and abiotic water column parameters were analyzed for comparison with growth rates among treatments. A second experiment was designed to determine foraging effects of shrimp on microbial and meiofaunal communities in replicate microcosm tanks. Microcosm tanks that were supplied with flow-through pond water were rapidly colonized by water-column and sediment microbes. Microbial and meiofaunal densities were monitored up to 20 days after initial colonization in the presence and absence of shrimp stocked at 40/m<sup>2</sup>. Results from these experiments indicate that shrimp growth is enhanced in the presence of water-column particles  $\geq 0.5 \mu$ m in size and to an even greater extent in the presence of particles  $\geq 5.0 \mu$ m. Shrimp growth rates were positively correlated with water-column particulate organic carbon, ATP, and algal cell density. Bacterial, algal, and protozoan densities were not affected by the presence of shrimp, whereas meiofaunal densities declined when shrimp were present. Enrichment of benthic food webs by water-column/sediment interactions may be important to shrimp growth even in intensive culture systems.

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## The Neustonic Myctophid Community: Are Lanternfishes Caught at the Surface Truly Neustonic?

STEWART B. REID<sup>45</sup>

The Hawaiian myctophid (lanternfish) fauna contains 10 species that are frequently caught on the ocean surface at night. Preliminary results suggest that the horizontal distributions of these nocturnally neustonic myctophids are complex, showing three distinct patterns and unresolved interactions with local advective processes. However, before their distributions can be fully addressed using purely neustonic sampling, it is necessary to establish that their populations are strictly limited to the neustonic habitat at night and, therefore, that neuston samples are representative. This paper discusses the results of a preliminary sampling transect off windward Oahu at five stations: over the 100-m, 250-m, and 500-m bottom isobaths, and at 50 and 100 nautical miles (92.5 and 185 km) offshore. Two designs of neuston net and an Issacs-Kidd midwater trawl (3-m mouth) were

utilized simultaneously, for a total of 79 neuston and 41 midwater samples, taken over a 13-day period. Results suggest that neustonic myctophids can be separated into four groups. "Strictly neustonic" species are limited to surface waters at night; "peripheral" species have an extended vertical distribution, which includes but is not limited to the surface (i.e., 0–200 m); "incidental" species are occasionally caught in neuston nets but are essentially midwater; "phototropic-midwater" species are frequently attracted to surface nightlights, but are essentially midwater. Neustonic sampling is a viable and representative method for the study of species considered "strictly neustonic." In Hawaiian waters this includes six species in two genera. These species will be used as subjects for further study of local myctophid distribution patterns.

## Cuttlebone Development in *Sepia officinalis*<sup>46</sup>

JENNIFER SALTZ<sup>47</sup>

Shell formation in molluscs is accomplished by accretionary growth. The shell is attached to the body by muscle attachments, which must translocate as the shell grows. Cephalopods, however, have an additional constraint on shell fabrication: they periodically form septa, so the muscles must completely detach and reattach in a forward position, a discontinuous process, as opposed to the gradual muscle translocation found in a snail or clam.

Because the shell of *Sepia officinalis* is internal, muscles are no longer necessary in attaching the animal to its shell. The muscle attachments may, however, play a role in the formation of septa (as is postulated in ammonites). I examined the development of the shell in relation to the process of septal fabrication in *Sepia officinalis* and *Spirula spirula* using scanning electron microscopy and serial sectioning. Structural differences and similarities between adjacent chambers were examined following the theoretical approach of Seilacher. My idea was to determine whether elements of the previous septum serve as a template for the next. In *Sepia*, the structural elements between the septa resemble the

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points of attachment between body whorl and fluted septa in fossil ammonites. Evidence of homology in the details of construction of

these elements in *Sepia* would support the theory that ammonites are ancestral to cuttlefish.

## **Transepithelial Transport of Organic Solutes by Cultured Fish Intestinal Cells<sup>48</sup>**

M. THAMOTHARAN<sup>49</sup>

Dispersed intestinal epithelial cells from the herbivorous teleost *Oreochromis mossambicus* were grown in modified medium 199 as confluent monolayers in primary culture on collagen-coated and uncoated 0.4- $\mu$ m porosity, 12-mm millipore millicells. Monolayers on coated filters exhibit an average electrical resistance of  $16.34 \pm 1.90$  ( $n = 6$ ) ohm  $\text{cm}^2$  and a transepithelial electrical potential of  $0.68 \pm 0.04$  mV ( $n = 6$ ) mucosa positive to serosa by 24 hr. Millicells containing confluent monolayers were inserted into Ussing chambers containing a complete physiological saline on both sides of the cell layer. Simultaneous mucosal to serosal (m to s) and serosal to mucosal (s to m) transepithelial fluxes of  $^3\text{H}$ - and  $^{14}\text{C}$ -labeled 0.1 mM D-glucose in one

series of experiments and 1.0 mM acetate in another were measured across these monolayers. Under control conditions, without any inhibitors, m to s glucose and acetate fluxes significantly exceeded the respective fluxes, s to m, providing net absorption of both organic solutes. Mucosal addition of 0.5 mM phloridzin or 5 mM unlabeled D-glucose reduced m to s glucose flux without influencing s to m movement of the sugar. Similarly, reduction of labeled acetate was also observed with mucosal addition of 10 mM unlabeled acetate. These results support the feasibility of using fish intestinal monolayers in primary culture for the study of transepithelial transport process.

## **Characteristics of Transepithelial Transport of Volatile Fatty Acids in the Intestine of an Herbivorous Teleost<sup>50</sup>**

ERIC TITUS<sup>51</sup>

Microbial fermentation is a symbiotic phenomenon found to occur in most classes of vertebrates and some invertebrates. Microbial degradation of plant celluloses and hemicelluloses in the gastrointestinal tract of the host results in the catabolic formation of nutritive volatile fatty acids (VFA: acetate,

propionate, butyrate). These VFAs are transported across the intestinal epithelium and further assimilated by the host organism. To date, this process has remained uninvestigated in fishes, which compose the largest class of vertebrates. The purpose of this study is to characterize the occurrence of fermentive processes in an herbivorous teleost, the African tilapia, *Oreochromis mossambicus*. These processes include both intraluminal generation of VFA in the intestine and transport of VFA across the intestinal wall. Intraluminal concentrations of VFA were determined using high-performance liquid

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chromatography. Results yielded concentrations of acetate in the gut ranging from 15 to 20 mM down the length of the intestine, as well as trace amounts of propionate. VFA transport was characterized using the isotopic tracer  $^3\text{H}$ -acetate in isolated membrane vesicles from both the mucosal (brush border) and serosal (basolateral) surfaces of the intestinal epithelial cells. A qualitatively identical facilitated exchange mechanism involving VFA and bicarbonate ( $\text{HCO}_3^-$ ) was found to occur at both membrane surfaces. When exposed to a profile of organic and inorganic anions, this

mechanism exchanged  $^3\text{H}$ -acetate specifically with either bicarbonate or other VFA. An apparent difference in kinetic parameters (mucosa:  $k_m = 6.44$  mM,  $V_{\max} = 5.30$  nmol Ac/mg protein/10 sec; serosa:  $k_m = 12.70$  mM,  $V_{\max} = 21.77$  nmol/mg protein/10 sec) suggests a facilitated exchange mechanism based on intra- and extracellular concentration differences of bicarbonate and VFA. Such a mechanism would prove to be energetically and nutritionally strategic in that it exchanges a cellular waste product (bicarbonate) for a potentially important anion.

### Diet, Food Habits, and Consumption in the Lemon Shark, *Negaprion brevirostris*<sup>52</sup>

BRAD WETHERBEE<sup>53</sup>

The lemon shark, *Negaprion brevirostris*, has been the subject of a series of studies examining characteristics of consumption and digestive processing of food. Diet of young lemon sharks and many other sharks is dominated by teleosts. Feeding by lemon sharks is asynchronous and intermittent and exhibits no pattern of periodicity. A meal is completely evacuated from the stomach of lemon sharks

25–41 hr after feeding, depending on meal type and temperature. Fecal production continues for 68–82 hr after feeding in the lemon shark. Lemon sharks absorb energy from food with an efficiency similar to that of most teleosts. Daily ration has been estimated at 1.5–2.1% body weight per day, which is intermediate in comparison to estimates for other species of sharks and is low in comparison to most teleosts. Lemon sharks are able to convert ingested energy to energy stored as growth as efficiently as many teleosts. Slow rates of digestion and consumption are factors that probably limit growth in the lemon shark and other elasmobranchs.

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